DEVELOPMENT OF SUITABLE WORKING PROTOCOL FOR IN VITRO TAPE STRIPPING: A CASE STUDY WITH BIOCOMPATIBLE ACECLOFENAC-LOADED TOPICAL NANOEMULSIONS

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ABSTRACT

Considering the numerous organizational and legislative issues associated with in vivo studies, the present study aimed to develop in vitro tape stripping protocol that could serve as a prospective technique for skin penetration studies. The research was mainly focused on the suitability of transepidermal water loss (TEWL) measurements, as a barrier integrity test for porcine ear skin subjected to freezing/thawing procedure, as well as on the selection of the most suitable device for pressing adhesive tapes onto the porcine ear surface during skin stripping procedure. Obtained results suggest that TEWL measurements were able to detect the damage of the stratum corneum (SC) caused by physical impairment (using adhesive tapes) and tissue degradation/dehydration (prolonged storage at –20ºC/ambient conditions). Penetration profiles of aceclofenac from nanoemulsions based on sucrose esters or polysorbate 80 as coemulsifiers, obtained in vitro (using a roller as pressure device), were in a good agreement with data obtained in vivo on humans, supporting the use of developed in vitro tape-stripping protocol in skin formulation development and optimization.

Keywords: tape stripping, porcine ear skin, nanoemulsions, in vitro-in vivo correlation, aceclofenac

INTRODUCTION

The rational approach for designing and optimizing novel skin formulations requires the use of well-defined method, which can enable the assessment of drug penetration into the skin. Among different techniques proposed, tape stripping stands out as a simple and minimally invasive technique for the assessment of drug dermal availability in vivo, in humans. However, considering that in vivo studies are frequently associated with numerous organizational and legislative issues (e.g. the need for complex documentation when applying for ethical committee approval, particularly for nanocarrier testing), the research efforts have been directed towards the validation of porcine ear skin as an alternative in vitro model for human studies [Klang, 2012; Leal, 2017]. Having in mind some unresolved issues, such as (i) the capability of applying TEWL measurements in assessing the integrity of the porcine ears subjected to freezing/thawing procedure and (ii) the selection of the most suitable device for pressing adhesive tapes onto the skin surface, the aim of present study was to develop the in vitro tape stripping working protocol that could be simply used in the averages laboratories, without the need for sophisticated equipment. Recently developed nanoemulsions intended for topical administration of aceclofenac (ACF) [Isailović, 2016] were utilized to assess the applicability of suggested protocol for the analysis of small variations in formulation composition on ACF delivery into the skin, by comparing with corresponding results obtained in vivo on humans.

RESEARCH CONCEPT

Development of in vitro tape stripping working protocol – evaluation of selected critical steps

Fresh porcine ears obtained from the local abattoir immediately after slaughter (before scalding), were washed under cold running water, blotted dry with the soft tissue, wrapped in the aluminum foil and stored at –20°C until use (within one to six months to evaluate the effect of storage duration on skin barrier integrity). On the day of experiment, after defrosting, hairs were shortened with scissors and when TEWL reached the values of approximately 15 $gm^{-2}h^{-1}$ (measured using a Tewameter® TM 210, Courage+Khazaka, Germany), ears were fixed on styrofoam plates [Klang, 2012]. In order to evaluate the capability of TEWL measurements to detect the changes in skin barrier

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function of defrosted porcine ears, periodic measurements of TEWL were performed during the progressive removal of the SC by adhesive tapes. Additionally, the TEWL measurements were performed on intact porcine ears periodically during 6 h of storage at ambient conditions, in order to detect the potential changes in skin barrier (due to tissue dehydration). Additionally, since application pressure is one of the basic factors influencing the amount of SC removed during the tape stripping procedure, two different methods (roller *vs*. weight of 300 g) were examined regarding the impact on TEWL and total quantity of ACF and SC removed with each tape.

In vitro tape stripping – a case study

In order to assess the applicability of developed protocol to detect the potentially meaningful differences in ACF penetration in the SC from nanoemulsions differing in the both, type and proportions of surfactants employed for stabilization (table 1), tape stripping was performed in vitro under infinite dose conditions and correlated with the results previously obtained in vivo on human volunteers [Isailović, 2016]. Briefly, when TEWL reached the values of ~15 $gm^{-2}h$, investigated formulations were carefully distributed on assigned skin sites. After a 2 h incubation period, the residual formulations were gently removed with dry cotton swabs, and 12 adhesive D-squame® discs (CuDerm Corporation, USA) were utilized for sequential removal of SC layers, after being submitted to uniform pressure using a roller device.

Table 1: Composition of investigated nanoemulsions, prepared using high pressure homogenization method

Excipients	Composition (% w/w)	
	$L_2P_2A/L_1S_1P_2A/L_{1.5}S_{0.5}P_2A$	L_2P80_2A
MCT*	10	10
Castor oil	10	10
Egg lecithin	2/1/1.5	2
Sucrose stearate	-/1/0.5	/
Sucrose palmitate	2	
Polysorbate 80	/	2
BHT*	0.05	0.05
Aceclofenac	1	1
Ultrapure water to	100	100

* MCT – medium chain triglycerides, BHT – butylhydroxytoluene

After removal of SC layers, each tape was transferred into a centrifuge tube and ACF was extracted with 4 mL of ethanol (70%, v/v). The tubes were sonicated (Sonorex RK 120H, Bandelin, Germany) for 15 min and then centrifuged at 4000 rpm for 5 min (Centrifuge MPW-56; MPW Med. Instruments, Poland). Supernatants were analyzed for ACF content using UHPLC-MS/MS method [Isailović, 2016]. Validation of the extraction procedure was performed by spiking tape-stripped samples of untreated SC with the known quantity of ACF dissolved in ethanol (70%, v/v). After solvent evaporation, the extraction procedure was carried out as described above and drug recovery was determined. It was found that ACF recovery from the SC was 95.8 ± 2.9%.

RESULTS

During tape stripping procedure, TEWL progressively increased as barrier function was perturbed by sequential removal of SC layers (figure 1). However, TEWL increased only slightly during the removal of 40% of the barrier's thickness, but changed more noticeably when more than 50% of SC was removed (removal of ~80% of SC was accompanied with ~5-fold increase of TEWL).

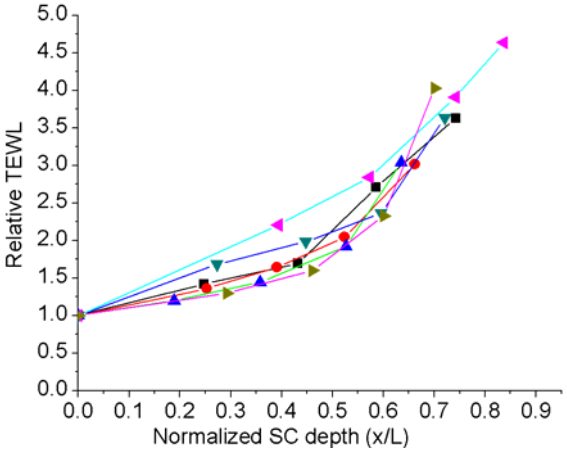

Figure 1: Relative TEWL (measured TEWL divided by pre-stripping value) as function of relative SC depth

Despite freezing/thawing procedure, the plots of 1/TEWL versus SC were highly linear for ears stored up to one month in the fridge (mean r^2 for six ears was 0.9723; representative plot is shown at figure 2), enabling the total thickness of SC (L) to be calculated as described previously using Fick's first law [Herkenne, 2006]. Interestingly, after three months (or more) storage of porcine ears at –20ºC, a linear correlation was deteriorated (r^2 = 0.8182, n=6), suggesting that prolonged storage impairs the barrier function of porcine ear skin.

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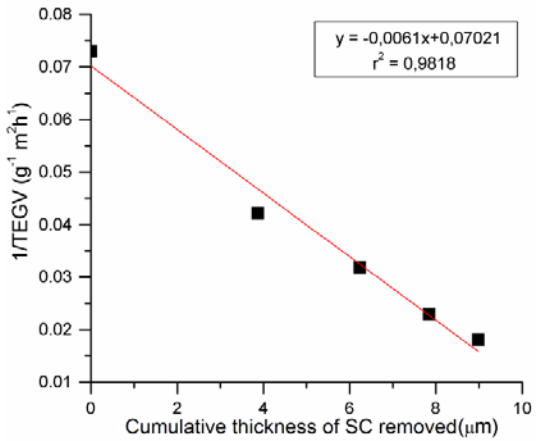

Figure 2: Representative linear plot of 1/TEWL as a function of the cumulative thickness of SC removed

Periodical TEWL measurements during 6 h-storage of porcine ears at ambient conditions revealed that TEWL values declined sharply 4 h after reaching the desired value of ~15 $gm^{-2}h^{-1}$, due to the tissue dehydration.

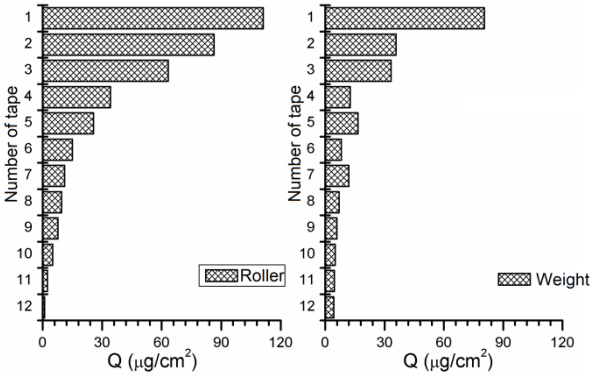

Figure 3: Quantity of ACF removed with each adhesive tape, reflecting the influence of different pressure device (roller *vs*. weight)

Further, TEWL values obtained during tape stripping performed using roller device were significantly higher compared to those obtained using weight (after 12th removed tape, TEWL was 54.1 and 37.2 $gm^{-2}h^{-1}$, respectively). Simultaneously, significantly higher thickness of SC was removed when roller was used to exert the pressure on the adhesive tape. Importantly, significantly higher amount of ACF was extracted from the SC when roller was used, clearly showing the gradual decrease of ACF quantity with increasing the number of removed tapes (figure 3).

Biocompatible aceclofenac-loaded nanoemulsions – a case study

ACF concentration versus relative SC depth profiles obtained after 2 h-application of investigated nanoemulsions on the porcine ear skin are shown in figure 4. Firstly, it could be seen that nanoemulsions based on sucrose esters as coemulsifiers enhanced cutaneous penetration of ACF, showing the higher drug levels at all depths into the SC in comparison with reference prepared with the polysorbate 80. However, due to high variability of obtained data, statistical significance was found only between $L_{1.5}S_{0.5}P_2A$ and L_2P80_2A formulations. Looking strictly at results obtained for sucrose esters-based nanoemulsions, investigated formulations could be ranked in the following order: $L_2P_2A < L_1S_1P_2A < L_{1.5}S_{0.5}P_2A$.

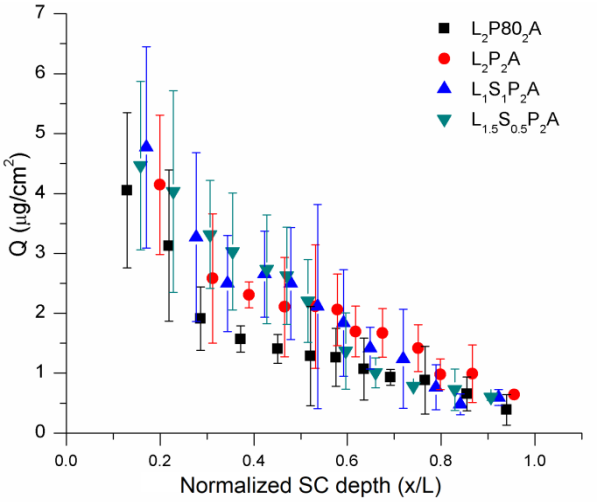

Figure 4: Comparative ACF penetration profiles across the SC path-length L (mean ± SD, n = 4)

Comparing the results obtained on porcine ears with results obtained in vivo, on human volunteers [Isailović, 2016], it could be noticed that results obtained in vitro are associated with significantly higher variability, probably due to difficulty in controlling the age, sex and way of pigs' nutrition.

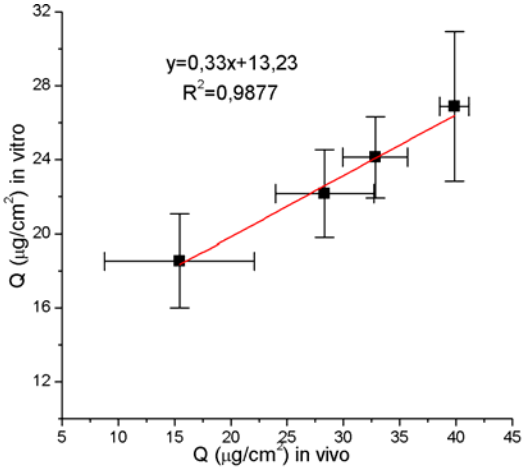

Figure 5: Correlation of the total amount of ACF recovered in the SC in vitro (porcine ear skin) and in vivo (human voluneteers)

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When the total quantity of ACF recovered in the SC in vitro 2 h after nanoemulsions application were plotted against the total amount of ACF inside the SC in vivo, a high correlation was observed (figure 5). Formulations with comparatively high amounts of ACF in the SC in vivo were associated with the greater penetration of ACF into the SC in vitro.

DISCUSSION

Despite the increased number of publications dealing with in vitro tape stripping, the literature data regarding the use of TEWL for skin barrier integrity assessment are quite controversial. The average SC thickness of defrosted porcine ears determined using TEWL measurements was 10.9 ± 3.2 μm, which was in accordance with literature data for SC thickness of fresh porcine ears (11.8 ± 4.0 μm) [Sekkat, 2002; Herkenne, 2006], proving the usefulness of TEWL measurement for skin barrier monitoring during tape stripping procedure in spite of freezing/thawing procedure. Furthermore, TEWL measurements enabled to detect changes in skin barrier function due to tissue degradation (caused by prolonged storage at –20ºC) and dehydration (caused by prolonged skin storage at ambient conditions). These findings indicate that porcine ears should be stored up to one month at –20ºC, simultaneously proving that the isolated porcine ear could be a suitable model for the short-time application studies [Klang, 2012].

Analyzing the literature data dealing with in vitro tape stripping technique, it can be observed that different devices were utilized to press the tapes onto the skin surface. Obtained results suggest that the roller is better option than weight of constant mass. During the rolling movement, it appears that the skin surface flattens, enabling the optimal adhesive bond of the tape with the skin surface [Herkenne, 2006].

Penetration profiles of ACF from developed nanoemulsions obtained in vitro using developed protocol, in spite of relatively high variability, were in a good agreement with previously published in vivo results, obtained under infinite dose conditions. Since all tested nanoemulsions exhibited the similar physicochemical parameters that are considered as a crucial for drug delivery into the skin (droplet size ~ 180 nm, zeta potential ~40 mV, pH ~3.5, viscosity ~3.5 mPa s), these findings confirmed that perturbation of the SC barrier by surfactants employed for nanoemulsion stabilization was primary mechanism responsible for observed differences in ACF skin penetration profiles. In the other words, it seems that sucrose esters are more efficient as skin penetration enhancers than conventionally used polyethoxylated surfactants such as polysorbate 80.

CONCLUSIONS

In vitro tape-stripping of developed protocol permits good prediction of drug uptake into the SC in vivo and therefore it may be highly useful in skin intended drug formulation development and optimization.

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REFERENCES

[Herkenne, 2006] Herkenne, C., Naik, A., Kalia, Y.N., Hadgraft, J., Guy, R.H., 2006, Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man, *Pharm. Res.*Vol. 23, pp. 1850–1856.

[Isailović, 2016] Isailović, T., Đorđević, S., Marković, B., Ranđelović, D., Cekić, N., Lukić, M., Pantelić, I., Daniels, R., Savić, S., 2016, Biocompatible nanoemulsions for improved aceclofenac skin delivery: formulation approach using combined mixture-process experimental design, *J. Pharm. Sci*. Vol. 105, pp. 308–323.

[Klang, 2012] Klang, V., Schwarz, J.C., Lenobel, B., Nadj M., Auböck, J., Wolzt, M., Valenta, C., 2012, In vitro vs. in vivo tape stripping: validation of the porcine ear model and penetration assessment of novel sucrose stearate emulsions, *Eur. J. Pharm. Biopharm*. Vol. 80, pp. 604–614.

[Leal, 2017] Leal, L.B., Cordery, S.F., Delgado-Charro, M.B., Bunge, A.L., Guy, R.H., 2017,. Bioequivalence methodologies for topical drug products: in vitro and ex vivo studies with a corticosteroid and an anti-fungal drug, *Pharm. Res*. Vol. 34, pp. 730–737.

[Sekkat, 2002] Sekkat, N., Kalia, Y.N,, Guy, R.H., 2002, Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo, *J. Pharm. Sci*. Vol. 9, pp. 2376–2381.

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